

WHAT IS CLAIMED IS:

1. A method of making a nucleic acid encoding a zinc finger protein (ZFP) comprising three contiguous zinc fingers domains, each separated from the other by no more than 10 amino acids,

(a) preparing a mixture, under conditions for performing a polymerase-chain reaction (PCR), comprising:

- (i) a first double-stranded oligonucleotide encoding a first zinc finger domain,
- (ii) a second double-stranded oligonucleotide encoding a second zinc finger domain,
- (iii) a third double-stranded oligonucleotide encoding a third zinc finger,
- (iv) a first PCR primer complementary to the 5' end of the first oligonucleotide,
- (v) a second PCR primer complementary to the 3' end of the third oligonucleotide,

wherein the 3' end of the first oligonucleotide is sufficiently complementary to the 5' end of the second oligonucleotide to prime synthesis of said second oligonucleotide therefrom,

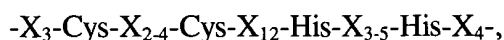
wherein the 3' end of the second oligonucleotide is sufficiently complementary to the 5' end of the third oligonucleotide to prime synthesis of said third oligonucleotide therefrom, and

wherein the 3' end of the first oligonucleotide is not complementary to the 5' end of the third oligonucleotide and the 3' end of the second oligonucleotide is not complementary to the 5' end of the first oligonucleotide;

(b) subjecting the mixture to a PCR; and

(c) recovering the nucleic acid encoding the three zinc finger domains and preparing a nucleic acid encoding said ZFP.

2. A method of making a nucleic acid encoding a zinc finger protein (ZFP) comprising three zinc fingers domains, each domain independently represented by the formula



and said domains, independently, covalently joined with from 0 to 10 amino acid residues

which comprises:

(a) preparing a mixture, under conditions for performing a polymerase-chain reaction (PCR), comprising:

- (i) a first double-stranded oligonucleotide encoding a first zinc finger domain,
- (ii) a second double-stranded oligonucleotide encoding a second zinc finger domain,
- (iii) a third double-stranded oligonucleotide encoding a third zinc finger,
- (iv) a first PCR primer complementary to the 5' end of the first oligonucleotide,
- (v) a second PCR primer complementary to the 3' end of the third oligonucleotide,

wherein the 3' end of the first oligonucleotide is sufficiently complementary to the 5' end of the second oligonucleotide to prime synthesis of said second oligonucleotide therefrom,

wherein the 3' end of the second oligonucleotide is sufficiently complementary to the 5' end of the third oligonucleotide to prime synthesis of said third oligonucleotide therefrom, and

wherein the 3' end of the first oligonucleotide is not complementary to the 5' end of the third oligonucleotide and the 3' end of the second oligonucleotide is not complementary to the 5' end of the first oligonucleotide;

(b) subjecting the mixture to a PCR; and

(c) recovering the nucleic acid encoding the three zinc finger domains and preparing a nucleic acid encoding said ZFP.

3. The method of Claim 2, wherein the first and second PCR primers independently include a restriction endonuclease recognition site.

4. The method of Claim 3, wherein said restriction endonuclease recognition site is for BbsI, BsaI, BsmBI, or BspMI.

5. The method of Claim 4, wherein said restriction endonuclease recognition site is for BsaI.

6. A method of making a nucleic acid encoding a zinc finger protein (ZFP) comprising four or more contiguous zinc fingers domains, each separated from the other by no more than 10 amino acids,

5 (a) preparing a first nucleic acid according to the method of Claim 3, wherein said second PCR primer includes a first restriction endonuclease recognition site;

(b) preparing a second nucleic acid according to the method of Claim 3, wherein said first and second PCR primers are complementary to the 5' and 3' ends, respectively, of the number of zinc finger domains selected for amplification,

10 wherein said first PCR primer includes a restriction endonuclease recognition site that, when subjected to cleavage by its corresponding restriction endonuclease, produces an end having a sequence which is complementary to and can anneal to, the end produced when said second PCR primer of step (a) is subjected to cleavage by its corresponding restriction endonuclease and

15 wherein said second PCR primer of step (b), optionally, includes a second restriction enzyme recognition site that, when subjected to cleavage produces an end that differs from and is not complementary to that produced from the first restriction endonuclease recognition site;

(c) optionally, preparing one or more additional nucleic acids by the method of Claim 3, wherein said first and second PCR primers are complementary to the 5' and 3' ends, respectively, of the number of zinc finger domains selected for amplification,

20 wherein said first PCR primer for each additional nucleic acid includes a restriction endonuclease recognition site that, when subjected to cleavage by its corresponding restriction endonuclease, produces an end having a sequence which is complementary to and can anneal to the end produced when the second PCR primer used for preparation of the second nucleic acid, or for the additional nucleic acid that is immediately upstream of the additional nucleic acid, is subjected to cleavage by its corresponding restriction endonuclease, and

25 wherein said second PCR primer for each additional nucleic acid, optionally, includes a restriction endonuclease recognition site that, when subjected to cleavage produces an end that differs from and is not complementary to any previously used;

(d) cleaving said first nucleic acid, said second nucleic acid and said additional nucleic acids, if prepared, with their corresponding restriction endonucleases to produce cleaved first, second and additional, if prepared, nucleic acids; and

5 (e) ligating said cleaved first, second and additional, if prepared, nucleic acids to produce the nucleic acid encoding a zinc finger protein (ZFP) having four or more zinc fingers domains.

7. A method of making a nucleic acid encoding a zinc finger protein (ZFP) having four or more zinc fingers domains, each domain independently represented by the formula

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$$-X_3-Cys-X_{2-4}-Cys-X_{12}-His-X_{3-5}-His-X_4-$$

and said domains, independently, covalently joined with from 0 to 10 amino acid residues which comprises:

(a) preparing a first nucleic acid according to the method of Claim 3, wherein said second PCR primer includes a first restriction endonuclease recognition site;

15 (b) preparing a second nucleic acid according to the method of Claim 3, wherein said first and second PCR primers are complementary to the 5' and 3' ends, respectively, of the number of zinc finger domains selected for amplification,

wherein said first PCR primer includes a restriction endonuclease recognition site that, when subjected to cleavage by its corresponding restriction endonuclease, produces an end
20 having a sequence which is complementary to and can anneal to, the end produced when said second PCR primer of step (a) is subjected to cleavage by its corresponding restriction endonuclease and

wherein said second PCR primer of step (b), optionally, includes a second restriction enzyme recognition site that, when subjected to cleavage produces an end that differs from and
25 is not complementary to that produced from the first restriction endonuclease recognition site;

(c) optionally, preparing one or more additional nucleic acids by the method of Claim 3, wherein said first and second PCR primers are complementary to the 5' and 3' ends, respectively, of the number of zinc finger domains selected for amplification,

wherein said first PCR primer for each additional nucleic acid includes a restriction
30 endonuclease recognition site that, when subjected to cleavage by its corresponding restriction

endonuclease, produces an end having a sequence which is complementary to and can anneal to the end produced when the second PCR primer used for preparation of the second nucleic acid, or for the additional nucleic acid that is immediately upstream of the additional nucleic acid, is subjected to cleavage by its corresponding restriction endonuclease, and

5 wherein said second PCR primer for each additional nucleic acid, optionally, includes a restriction endonuclease recognition site that, when subjected to cleavage produces an end that differs from and is not complementary to any previously used;

(d) cleaving said first nucleic acid, said second nucleic acid and said additional nucleic acids, if prepared, with their corresponding restriction endonucleases to produce cleaved first, 10 second and additional, if prepared, nucleic acids; and

(e) ligating said cleaved first, second and additional, if prepared, nucleic acids to produce the nucleic acid encoding a zinc finger protein (ZFP) having four or more zinc fingers domains.

15 8. The method of Claim 6 or 7, wherein each restriction endonuclease is, independently, BbsI, BsaI, BsmBI, or BspMI, and each endonuclease produces a unique pair of cleavable, anneable ends.

20 9. The method of Claim 6 or 7, wherein the restriction endonuclease is BsaI and each use thereof produces a unique pair of cleavable, anneable ends.

10. The method of Claim 6 or 7, wherein step (c) is omitted and said nucleic acid encoding a zinc finger protein (ZFP) has four, five or six zinc finger domains.

25 11. The method of Claim 10, wherein said restriction endonuclease is BbsI, BsaI, BsmBI, or BspMI.

12. The method of Claim 10, wherein said restriction endonuclease is BsaI.

13. The method of Claim 6 or 7, wherein the PCR primers for the second nucleic acid were selected to amplify three zinc finger domains, one additional nucleic acid is prepared by step (c), and said nucleic acid encoding a zinc finger protein (ZFP) has seven, eight or nine zinc finger domains.

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14. The method of Claim 13, wherein each restriction endonuclease is, independently, BbsI, BsaI, BsmBI, or BspMI, and each endonuclease produces a unique pair of cleavable, anneable ends.

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15. The method of Claim 13, wherein the restriction endonuclease is BsaI and each use thereof produces a unique pair of cleavable, anneable ends.

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16. The method of any one of claims 1, 2, 6 or 7, wherein the sequences of said oligonucleotides are selected to provide for optimal codon usage for an organism.

17. The method of Claim 16, wherein said organism is a bacterium, a fungus, a yeast, an animal, an insect or a plant.

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18. The method of Claim 17, wherein said bacterium is *E. coli*.

19. The method of Claim 17, wherein said animal is a human or a commercial animal.

20. The method of Claim 17, wherein said plant is a cereal plant.

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21. The method of Claim 17, wherein said plant is rice, tomato or corn.

22. The method of Claim 17, wherein said plant is a transgenic plant.

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23. An expression vector comprising a nucleic acid prepared by the method of any one of Claims 1, 2, 6 or 7.

24. A host cell comprising the expression vector of Claim 23.

25 A method of preparing a zinc finger protein which comprises

5 (a) culturing the host cell of Claim 24 for a time and under conditions to express said ZFP; and

(b) recovering said ZFP.

10 26. The method of Claim 10, wherein the sequences of said oligonucleotides are selected to provide for optimal codon usage for an organism.

27. The method of Claim 26, wherein said organism is a bacterium, a fungus, a yeast, an animal, an insect or a plant.

15 28. An expression vector comprising a nucleic acid prepared by the method of Claim 10.

29. A host cell comprising the expression vector of Claim 28.

20 30. A method of preparing a zinc finger protein which comprises

(a) culturing the host cell of Claim 29 for a time and under conditions to express said ZFP; and

(b) recovering said ZFP.

25 31. The method of Claim 13, wherein the sequences of said oligonucleotides are selected to provide for optimal codon usage for an organism.

32. The method of Claim 31, wherein said organism is a bacterium, a fungus, a yeast, an animal, an insect or a plant.

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33. An expression vector comprising a nucleic acid prepared by the method of Claim 13.

34. A host cell comprising the expression vector of Claim 33.

35. A method of preparing a zinc finger protein which comprises
(a) culturing the host cell of Claim 34 for a time and under conditions to express said ZFP; and
(b) recovering said ZFP.

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